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# CLASSIFICATION OF THE BACILLUS WELCHII GROUP OF BACTERIA \*

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The name, *B. welchii*, represents a fairly well-differentiated group of organisms which have the following characteristics in common: They are large, anthrax-like bacilli with slightly rounded ends, non-motile, and gram-positive; spore formation is not constant in artificial media, and occurs only in neutral or alkaline media in the absence of fermentable sugar; they cause stormy fermentation of milk with the production of butyric acid, and may or may not slowly liquefy plain or sugar-free gelatin; they ferment, with production of acid and gas, all of the monosaccharids and disaccharids.

The group is thus differentiated, on the one hand, from the motile butyric acid bacilli, such as the bacillus amylobacter of Gruber and of Bredemann, which is motile, forms spores in milk, and contains granules which stain blue with iodine; and, on the other hand, from the so-called "putrefactive butyric acid bacilli," such as the bacillus chauvei, which do not cause stormy fermentation of milk, but form spores readily in most media.

Numerous strains of bacteria identical with, or closely related to, the bacillus welchii, under a number of names, have been described by different writers. At least eight diverse names have been applied: The bacillus of acute articular rheumatism (Achalme,<sup>1</sup> 1891); *B. aerogenes capsulatus* (Welch and Nuttall,<sup>2</sup> 1892); *B. phlegmonis emphysematosae* (Fraenkel,<sup>3</sup> 1893); *B. enteritidis sporogenes* (Klein,<sup>4</sup> 1895); *Bacillus perfringens* (Veillon and Zuber,<sup>5</sup> 1898); *B. vaginae emphysematosae* (Lindenthal,<sup>6</sup> 1897); *B. cadaveris butyricus* (Buday,<sup>7</sup> 1898); *Granulo-bacillus saccharobutyricus liquefaciens immobilis* (Schattenfroh and Grassberger,<sup>8</sup> 1899).

While all of these organisms evidently belong to the same group of bacteria, they show certain minor differences among themselves. Heretofore, no systematic attempt has been made to classify the organisms within the bacillus

\* Received for publication September 22, 1914.

1. Compt. rend. Soc. de biol., 1891, 43, p. 651.
2. Johns Hopkins Hosp. Bull., 1892, 3, p. 81.
3. Ueber Gasphegmone, Hamburg and Leipzig, 1893.
4. Centralbl. f. Bakteriologie, 1895, 18, p. 737.
5. Arch. de méd. expér. et d'anat. path., 1898, 10, p. 517.
6. Wien. klin. Wchnschr., 1897, 10, p. 3.
7. Centralbl. f. Bakteriologie, 1898, 24, p. 369.
8. Ibid., Abt. 2, 1899, 5, pp. 209, 697; Arch. f. Hyg., 1900, 37, p. 54.

welchii group. In other studies on this group, it was found both convenient and desirable to attempt to separate the various strains into subgroups. But before presenting the results of this work, it seems advisable to present briefly the conclusions reached by others.

Fraenkel<sup>9</sup> and Klein<sup>10</sup> recognized only one variety of the organisms isolated by them, namely, the pathogenic. Klein considered the non-pathogenic form identical with *B. butyricus* of Botkin.<sup>11</sup> Schattenfroh and Grassberger<sup>12</sup> and Passini<sup>13</sup> differentiated two species, virulent and non-virulent.

Hitschmann and Lindenthal<sup>14</sup> were unable, either from the behavior in the animal body or in cultures, to differentiate varieties. They found tests of pathogenicity especially unreliable as a means of differentiation. Passini,<sup>15</sup> Werner<sup>16</sup> and Rocchi<sup>17</sup> attempted to distinguish subvarieties by means of serological reactions, but without success. Herter<sup>18</sup> believed that there are subvarieties of *B. welchii* "based mainly on differences respecting the difficulty of sporulation, upon pathogenic qualities, hemolytic properties, indol production, rapidity of gas formation in man and animals, etc." He reported no results of any attempt to apply these factors as a basis of classification. Rosenthal<sup>19</sup> differentiated two varieties—"variete rheumatismale" and "variete banale." His basis of classification, however, is not entirely convincing.

Jackson<sup>20</sup> described two types of the bacillus welchii. Type "A" was non-motile, produced 26 percent gas in raffinose broth and 10 percent gas in mannite broth, the reaction remaining neutral in each case, and produced 92 percent gas in lactose bile broth. Type "B" was motile, produced 22 percent gas in raffinose broth, the reaction becoming acid, and 56 percent gas in mannite broth, the reaction remaining neutral, and produced no gas in lactose bile broth.

Attempts to subdivide this group must be made only under the most exactly uniform conditions. Slight differences in conditions will disclose what appears to be a disconcerting tendency on the part of these organisms to display remarkable variations in the manner of growth and the degree and vigor of activity. For instance, depending upon the amount of free oxygen present in milk, one may find, after 24-48 hours' incubation, either no change in the medium, or coagulation without gas formation, coagulation with very slight gas formation, or typical stormy fermentation even to the extent of blowing out the cotton plug in the tube.

The present attempt at classification is based upon a study of some fifty strains isolated from various sources, the most scrupulous care being exercised to obtain pure cultures. Complete records of only

9. Ztschr. f. Hyg. u. Infektionskrankh., 1902, 40, p. 73.

10. Ann. Rep. Med. Off. Loc. Govt. Bd., 1901-02, p. 404.

11. Ztschr. f. Hyg. u. Infektionskrankh., 1894, 11, p. 421.

12. Arch. f. Hyg., 1900, 37, p. 54.

13. Ztschr. f. Hyg. u. Infektionskrankh., 1905, 49, p. 135.

14. Sitzungsber. d. k. Akad. d. Wissensch., Math.-Naturwiss. Klasse, 1899, 108, p. 67.

15. München. med. Wehnschr., 1904, 51, p. 1283.

16. Arch. f. Hyg., 1905, 53, p. 128.

17. Centralbl. f. Bakteriöl., Orig., 1911, 60, p. 174.

18. Harvey Society Lectures, 1906-07, p. 64.

19. Centralbl. f. Bakteriöl., Ref., 1909, 44, p. 609.

20. Personal Communication sent to bacteriologists in the United States, 1912.

thirty organisms were kept, the others being lost. These thirty strains were obtained from the following sources: from human stools, 19 (normal stools of adults, 2; diarrheal stools of adults, 1; normal stools of infants, 5; diarrheal stools of infants, 4; stools of patients with pernicious anemia, 5; stools of patients with typhoid fever, 2); from the soil, 2; from sewage, 1; from milk, 1; from bird feces, 1; from cow feces, 3; from the lumen of a normal appendix obtained at autopsy, 1; from the washings from vegetables (potatoes and lettuce) bought in open market, 2.

The basis of the proposed classification is the fermentation reaction of the bacillus welchii in inulin and glycerin broths and its ability to produce spores in neutral media containing these substances. Because of the well-known ability of this organism to produce gas from sugar-free broth, the mere presence of gas cannot be taken as an indication of the ability of a given strain to attack inulin, glycerin, or other substances in such broth. In these experiments, it was considered that the organism had attacked the carbohydrate only when there was a definite increase of acidity along with the production of gas. In no instance was there more than 20 percent of gas when there was no increase in acidity. Even in the presence of a small amount of gas, if there was no increase in acidity of the medium and especially if spores were found to be present, it was believed that the strain in question had not acted upon the carbohydrate.

Upon the basis of their action upon glycerin and inulin broths, organisms belonging to the bacilli welchii group may be divided into four subgroups:

*Subgroup 1.*—Ferment both inulin and glycerin with production of gas and increase of acidity. Do not form spores in media containing either substance. Produce strong hemolysins and are pathogenic for guinea-pigs, even after many months' cultivation upon artificial media.

*Subgroup 2.*—Produce acid and gas from glycerin but not from inulin. Form spores in inulin, but not in glycerin broth. Hemolytic and pathogenic powers variable.

*Subgroup 3.*—Produce acid and gas from inulin but not from glycerin. Form spores in glycerin but not in inulin broth. Hemolysis and pathogenicity variable.

*Subgroup 4.*—Do not produce acid or gas from either inulin or glycerin, and form spores in both inulin and glycerin broths.

The source of culture gives no indication as to the subgroup to which it belongs, as shown by the grouping of the following twenty strains:

*Subgroup 1.*—Four strains. Normal stool, adult; diarrheal stool, adult; normal stool, three-day-old infant; diarrheal stool, infant.

*Subgroup 2.*—Seven strains. Soil, Brenham, Texas; normal stool, six-day-old infant; stool, adult with pernicious anemia; cow feces — three specimens; milk.

*Subgroup 3.*—Five strains. Normal stool, adult; stool, adult with pernicious anemia; stool, adult with typhoid fever; diarrheal stool, infant; normal appendix, autopsy.

*Subgroup 4.*—Four strains. Boston street dust; stools, patients with pernicious anemia — three specimens.

While the source of a culture does not determine the group to which it belongs, there may be some significance in certain facts shown in the tabulation: (1) All the strains isolated from cow feces and the one isolated from milk belonged to Subgroup 2. (2) Of the strains isolated from diarrheal stools, none belonged to Subgroup 2. The facts here presented are too few to justify a positive statement, but the possibility may be suggested that the variety of *Bacillus welchii* found in the intestinal tract of cows and in milk is not capable of causing gas bacillus diarrhea. If this is true, the human gas bacillus carrier assumes a more serious importance in the spread of this infection. (3) The strains of the *Bacillus welchii* from the stools of patient with pernicious anemia tend to fall into Subgroup 4.

#### SUMMARY

The fermentation reactions in and the ability or inability to form spores in glycerin and inulin broths appear to furnish a reasonable and dependable means of dividing bacteria of the *Bacillus welchii* group into four subgroups.

The source of a culture is no indication of the subgroup to which it belongs. It may be significant that all of the strains isolated from cow feces belonged to a group which contained none of those isolated from diarrheal stools. In view of this, it is suggested that the human gas bacillus carrier may prove to be of greater importance in the spread of this infection than milk infected with this organism from bovine sources.

The majority of those strains isolated from the stools of patients with pernicious anemia belonged to the same subgroup.